

Review Article

PARP1: Structural insights and pharmacological targets for inhibition

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ABSTRACT

Poly(ADP-ribose) polymerase 1 (PARP1, also known as ADPR1) is a multifunctional human ADP-ribosyltransferase. It plays a role in multiple DNA repair pathways, including the base excision repair (BER), non-homologous end joining (NHEJ), homologous recombination (HR), and Okazaki-fragment processing pathways. In response to DNA strand breaks, PARP1 covalently attaches ADP-ribose moieties to arginine, glutamate, aspartate, cysteine, lysine, and serine acceptor sites on both itself and other proteins. This signal recruits DNA repair proteins to the site of DNA damage. PARP1 binding to these sites enhances ADP-ribosylation via allosteric communication between the distant DNA binding and catalytic domains. In this review, we provide a general overview of PARP1 and emphasize novel potential approaches for pharmacological inhibition. Clinical PARP1 inhibitors bind the catalytic pocket, where they directly interfere with ADP-ribosylation. Some inhibitors may further enhance potency by “trapping” PARP1 on DNA via an allosteric mechanism, though this proposed mode of action remains controversial. PARP1 inhibitors are used clinically to treat some cancers, but resistance is common, so novel pharmacological approaches are urgently needed. One approach may be to design novel small molecules that bind at inter-domain interfaces that are essential for PARP1 allostericity. To illustrate these points, this review also includes instructive videos showing PARP1 structures and mechanisms.

1. Introduction

Poly(ADP-ribose) polymerases (PARPs) comprise a family of ADP-ribosyltransferases found in all six major eukaryotic supergroups, as well as several species of prokaryotes and dsDNA virus [1]. PARP proteins transfer ADP-ribose moieties to arginine, glutamate, aspartate, cysteine, lysine, and serine acceptor sites [2–6].

Among the 17 PARP proteins in humans, poly(ADP-ribose) polymerase 1 (PARP1, also known as ADPR1) was the first identified [7,8]. PARP1 is a multifunctional protein that initiates DNA repair [9,10]. Given its biological importance and potential as a cancer drug target, PARP1 has been the subject of intense study (>1200 publications since 2015 according to PubMed). This review aims to provide a general overview of PARP1, with appropriate references to domain-specific

articles for those who wish to learn more. We also include helpful videos [11] illustrating PARP1 structures and mechanisms that others can use in their own presentations and classroom lectures.

We will first provide a broad overview of four DNA repair pathways relevant to PARP1: the base excision repair (BER), non-homologous end joining (NHEJ), homologous recombination (HR), and Okazaki-fragment processing pathways. We will then describe PARP1's role in recruiting repair pathways to DNA strand breaks, as well as the orthosteric/allosteric mechanisms that ultimately enable DNA repair. Next, we will review the relevant pharmacology, including PARP1's role in HR-deficient cancer, clinically approved small-molecule PARP1 inhibitors (PARPi), and known PARPi resistance mechanisms. Finally, we will discuss the future of PARP1 inhibition.

Abbreviations: AD, automodification domain; AP, apurinic/apyrimidinic; ART, ADP-ribosyl transferase; ASL, α D-active site loop; BAD, benzamide adenine dinucleotide; BER, base excision repair; BRCT, BRCA C-terminus; CAT, catalytic; DBD, DNA binding domain; DDR, DNA damage response; DSB, double strand DNA break; HD, helical domain; HR, homologous recombination; MARYlation, mono-ADP-ribosylation; MMEJ, microhomology mediated end joining; NAD+, nicotinamide adenine dinucleotide; NHEJ, non-homologous end joining; PAR, poly(ADP-ribose); PARP, poly(ADP-ribose) polymerase; PARP1, poly(ADP-ribose) polymerase 1; PARPi, PARP1 inhibitors; PARylation, poly-ADP-ribosylation; SSB, single strand DNA break; WGR, tryptophan-glycine-arginine-rich; Zn1, zinc finger 1; Zn2, zinc finger 2; Zn3, zinc finger 3.

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2. PARP1 and DNA damage repair

At least four DNA repair pathways are influenced, to one degree or another, by PARP1 activity. Collectively, pathways such as these constitute the DNA damage response (DDR) [12–14]. DNA integrity is continuously threatened by environmental agents, reactive oxygen species, UV radiation, and replication errors [14,15], which introduce base modifications, abasic sites, double strand DNA breaks (DSBs), and single strand DNA breaks (SSBs). During the process of normal DNA replication, unligated Okazaki fragments on the lagging strand also contain transient SSBs [16]. If unrepaired, this damage can result in cancer-causing mutations, cell death, or cellular senescence. Life has evolved multiple strategies for identifying different types of DNA damage, as required to repair or terminate the cell [12,13]. In humans, prominent DDR mechanisms include the BER, NHEJ, HR, and Okazaki-fragment processing pathways.

2.1. Base excision repair (BER)

PARP1 recruits BER repair proteins to DNA strand breaks undergoing processing [17] and so plays a major role in the BER pathway. The BER mechanism repairs many types of DNA lesions, including abasic sites, SSBs, and base alterations caused by oxidation, alkylation, or deamination [17–19]. Unless corrected, these lesions can stall DNA replication and transcription [20], cause genomic instability, progress into DSBs [21], and result in cell death [20]. As its name implies, BER excises small regions of DNA damage, thereby creating an SSB intermediate [19]. Subsequent proteins in the BER pathway protect this intermediate from progressing into a DSB and ensure that the damage is repaired [18,19].

Several variations of the BER pathway share key proteins in common. All follow five general steps [18]: (1) a damage-specific glycosylase detects and excises the damaged DNA base, producing an apurinic/apyrimidinic (AP), or abasic, site; (2) incise the AP site (creating a SSB) using an AP endonuclease or a glycosylase-associated AP lyase; (3) repair the terminal ends of the sugar backbone using a lyase or a phosphodiesterase; (4) replace the missing region with complementary DNA using a DNA polymerase; and (5) ligate the DNA to remove nicks using a DNA ligase. A more detailed review of the BER pathway can be found in refs. [18,22].

2.2. Non-homologous end joining (NHEJ)

PARP1 binding and activation also impact NHEJ, which repairs DSBs by direct ligation [23]. Though this repair mechanism preserves DNA integrity generally, NHEJ is error prone because the rejoined ends often share little to no homology. The lack of an effective guide results in lost DNA bases at the joining site [23].

The role of PARP1 in the canonical NHEJ (c-NHEJ) sub-pathway is surprisingly uncertain. c-NHEJ is activated when Ku70 and Ku80 (Ku proteins) bind to sites of DSBs [21]. PARP1 may play a role in recruiting proteins such as these to the locations of DNA strand breaks; PARP1 covalently attaches adenosine diphosphate ribose (ADP-ribose) chains to DNA repair proteins via poly-ADP-ribosylation (PARylation, see below), and Ku proteins are known to bind these chains [21,23,24]. On the other hand, PARP1 and Ku compete with each other for DSB binding, so PARP1 may also inhibit c-NHEJ [25].

The role of PARP1 in the more recently discovered alternative NHEJ (alt-NHEJ) sub-pathway is less ambiguous: its binding and activation stimulates alt-NHEJ [23]. Given that Ku proteins have stronger affinities for DSB sites, PARP1-initiated alt-NHEJ likely serves as a backup to Ku-initiated c-NHEJ [25]. Indeed, this sub-pathway has been called “backup NHEJ,” as well as microhomology mediated end joining (MMEJ) [23]. More detailed reviews of NHEJ are provided in refs. [26, 27].

2.3. Homologous recombination (HR)

PARP1 does not directly stimulate the HR pathway, but cells that are HR deficient tend to be hypersensitive to PARP1 inhibition [28–31]. HR uses homologous DNA as a template for DSB repair [21,26]. Unlike NHEJ, which remains active throughout the cell cycle, HR activity is mostly limited to the S and G2 phases [13,26,32]. Additionally, HR repair is less error prone than NHEJ [21,26] and is therefore critically important for genome stability and repair fidelity, particularly during DNA replication [32].

PARP1 inhibitors have been successfully used to treat patients with HR-deficient cancers [28–31]. Many cancer cells have deficiencies in HR-pathway proteins [13,32–34], especially the breast-cancer tumor-suppressor proteins BRCA1 and BRCA2 [13,32]. These proteins are involved in transcription regulation [33], end resection [32,34], filament promotion [32,34], DNA-strand protection at stalled forks [32], cell-cycle checkpoints [33], and cellular apoptosis [33]. HR-deficient cells must rely more heavily on other repair pathways such as BER or NHEJ to avoid genomic instability [13,34,35]. Therefore, HR-deficient cancers are less able to survive further loss of non-HR repair pathways (e.g., due to PARP1 inhibition) than are HR-proficient cells [13,34,35]. More detailed reviews of HR repair and BRCA proteins can be found in refs. [32,36].

2.4. Okazaki-fragment processing

During DNA replication, the replisome machinery unwinds DNA, separating the two complementary strands at the so-called replication fork [37]. On the leading strand, replication occurs continuously in the 5' to 3' direction, towards the progressing fork. But the complementary (lagging) strand runs in the 3' to 5' direction, so replication must proceed discontinuously in short segments with RNA primers in the direction opposite the fork. These short segments, called Okazaki fragments [38,39], must be processed, and the resulting SSBs must then be ligated.

Recent work has found that PARP1 plays a critical role in detecting these SSBs, which occur appropriately during DNA replication rather than as a result of unexpected damage. Indeed, most PARP activity occurs at sites of DNA replication during S phase, where PARP1 likely serves to recruit SSBs repair proteins such as XRCC1 [16].

3. PARP1 recruits DNA repair proteins via ADP-ribosylation

In this section, we describe how PARP1-mediated ADP-ribosylation recruits DNA repair proteins to strand breaks. We discuss ADP-ribosylation generally, as well as PARP1's role in ADP-ribose-mediated signaling.

3.1. ADP-ribosylation, a form of DNA repair signaling

Nicotinamide adenine dinucleotide (NAD⁺) is an organic small molecule common to all living organisms [2]. It is composed of an adenosine 5'-monophosphate (ADP) and a nicotinamide mononucleotide that are covalently connected by a phosphodiester bond (Fig. S1) [2,40]. Among its many biological roles, NAD⁺ can be catabolized to ADP-ribose and nicotinamide via cleavage of the N-glycosidic bond (Fig. S1) [2].

ADP-ribosyltransferases catalyze this reaction and attach the resulting ADP-ribose molecule(s) to acceptor protein(s) [2]. Both mono-ADP-ribosylation (MARylation) and poly-ADP-ribosylation (PARylation) are possible [2]; the former involves a single ADP-ribose addition, and the latter produces both linear and branching poly (ADP-ribose) (PAR) chains (Fig. S1B) [2]. In contrast, proteins such as PAR glycohydrolase and PAR hydrolase [5,34] rapidly degrade these chains. Given that ADP-ribosylation consumes both ATP and NAD⁺, a proper balance between PAR-chain synthesis and degradation is critical. Both hyperactivation of PARP1 (leading to increased ADP-ribosylation)

and inhibition of PAR glycohydrolase (leading to impaired PAR-chain degradation) can cause ATP and NAD⁺ depletion, leading to a form of metabolic death known as parthanatos [24,34,41,42].

PAR chains act as scaffolds that recruit DNA repair proteins to the locations of DNA strand breaks [21,43]. Many DNA repair proteins contain a PAR-binding motif that interacts non-covalently with PAR-chains, including p53, DNA ligase III, XRCC1, DNA-PK(CS), and Ku70 [44]. PAR-binding motifs are typically 20 amino acids long and consist of two conserved regions: a region dense in basic amino acids, and a region consisting of both hydrophobic and basic amino acids [44]. The location of PAR-binding motifs coincides with five different functional domains that are involved in nuclear localization, protein degradation, nuclear export, protein-protein interactions, and DNA binding [44]. Several other structures also facilitate protein-PAR non-covalent interactions, including the PAR-binding zinc fingers found in the NHEJ protein aprataxin and PNK-like factor (APLF) [2,45], tryptophan and glutamic-acid rich WWE domains found in E3 ligases [2,46], and macro domains found in PAR glycohydrolase [2,47,48].

3.2. PARP1 is both an ADP-ribosyltransferase and an ADP-ribose acceptor

Multiple members of the PARP family, including PARP1, act both as ADP-ribosyltransferases and PARylation acceptors [2]. Specifically, PARP1 ADP-ribosylates (1) itself as a monomer (in cis modification) [49]; (2) other PARP1 molecules, both as symmetric and asymmetric homodimers (in trans modification) [49]; and (3) other proteins [49] such as XRCC1, XRCC5, XRCC6, DNA topoisomerase 2-alpha (TOP2), replication protein A1 (RPA1), and RPA2 [50].

The primary acceptor of PARP1-mediated PARylation is PARP1 itself [5,51]. There are at least 102 potential PAR-acceptor sites on PARP1 [3, 52–58] available for autoPARylation (Fig. S2) [2,13,40,49]. The PARP1 automodification domain (AD) houses many of the most

well-characterized autoPARylation sites [3,5,51,53–58]. This domain includes the BRCA C-terminus (BRCT) subdomain, which interacts with other proteins (e.g., the BER protein XRCC1 [59,60]) but is not essential for DNA binding or catalytic activity (Fig. 1, Video S1) [40,51]. Aside from serving as a scaffold for recruiting DNA repair proteins to strand breaks [21,43], negatively charged PAR chains attached to PARP1 may also help PARP1 dissociate from negatively charged DNA [34], allowing recruited DNA repair-proteins to access the damage site [61].

4. PARP1 allosteric and catalytic mechanisms

In this section, we will discuss how PARP1 binds to DNA strand breaks, how that binding allosterically activates the catalytic mechanism, and how PARP1 eventually dissociates from the bound DNA. Although we here focus on the PARP1 response to DNA damage, we note that the protein plays other cellular roles as well. Interested readers may wish to consult refs. [62,63] to learn more about PARP1 and inflammation, as well as refs. [63,64] to learn more about PARP1 ADP-ribosylation of chromatin.

4.1. Recognizing and binding to DNA strand breaks

The PARP1 N-terminal DNA binding domain (DBD) includes three DNA binding zinc fingers that bind DNA strand breaks: zinc finger 1 (Zn1), zinc finger 2 (Zn2), and zinc finger 3 (Zn3) (Fig. 1, Video S1) [51, 64–66]. Though most zinc fingers recognize specific DNA sequences, the PARP1 zinc fingers are unusual in that they recognize DNA structures [5, 67]. Zn1 and Zn2 identify many types of DNA lesions with high affinity and are required for DNA binding [2,51,67,68]. Without Zn1, Zn2, and Zn3, a SSB cannot trigger PARP1 DNA-dependent catalytic activation [68–71]. Similarly, without Zn1 and Zn3, a DSB cannot trigger activation [2,51,66–68]. Simultaneous deletion of Zn1 and Zn2 reduces PARP1 binding affinity by over 250-fold, and a fragment containing only

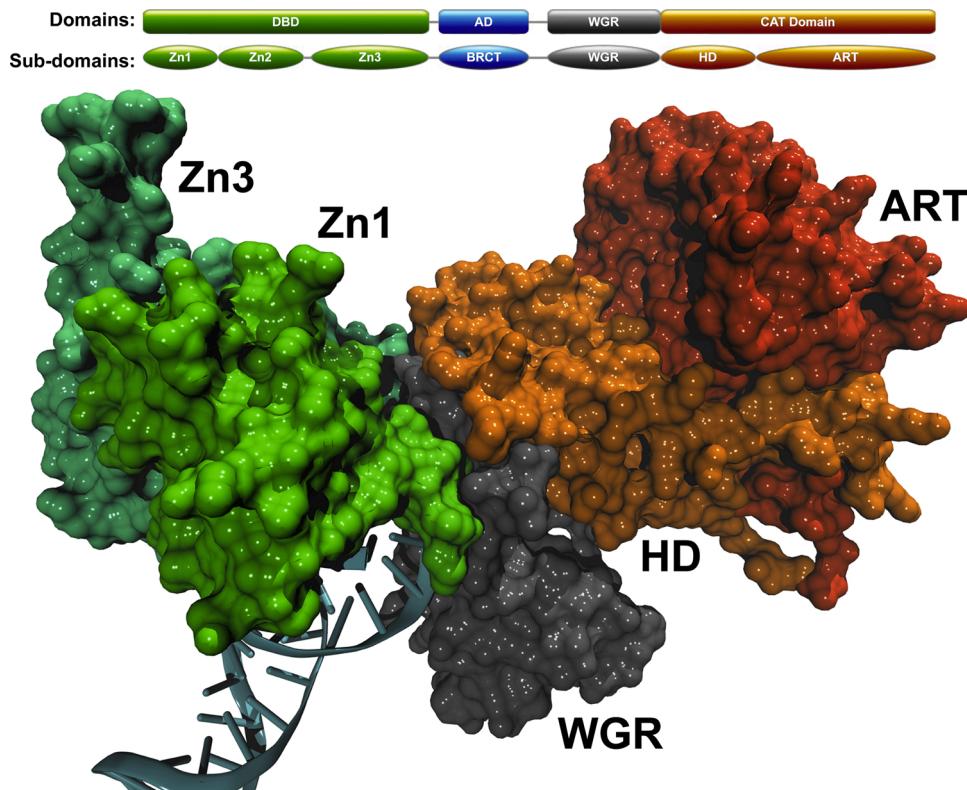


Fig. 1. PARP1 domains and subdomains. The protein schematic (above) was generated with the help of PROSITE [159]. The protein structure (below) shows PARP1 bound to DNA (PDB: 4DQY). The zinc finger 2 (Zn2) and BRCA C-terminus (BRCT) subdomains are not shown, as they are not present in the crystal structure. See Video S1 for an additional review of these PARP1 domains.

Zn1 and Zn2 binds to DNA with nearly the same affinity as wild-type PARP1 [67]. Isolated Zn1 fragments have much weaker binding affinities for DNA than do isolated Zn2 [67]. But Zn2 deletion does not impact the DNA binding affinity of PARP1, and Zn1 deletion results in an approximately three-fold reduction in binding affinity [67]. Despite that fact that Zn2 has a much stronger affinity for DNA, Zn1 combined with the C-terminal region of the DBD can apparently compensate for the loss of Zn2 [67].

Zn3, which has a different structure and function than Zn1 and Zn2 [51,67,72], makes crucial inter-domain contacts that are required for PARP1 activation (Fig. 1, Video S1) [51,67]. Similarly, the tryptophan-glycine-arginine-rich (WGR) domain also interacts with DNA and contributes to important inter-domain contacts (Fig. 1, Video S1), as described below.

4.2. Allosteric communication between the DNA binding and catalytic domains

DNA binding induces cascading conformational changes that ultimately initiate PARP1-mediated ADP-ribosylation [64,73]. PARP1 catalytic activity is largely DNA dependent. When not bound to DNA, PARP1 has only a low basal level of DNA-independent catalytic activity. In contrast, PARP1 activity is much elevated when it binds DNA [5,51]. The minimum PARP1 construct with near-wild-type DNA-dependent activity (i.e., activity when bound to DNA) contains the Zn1, Zn3, WGR, and catalytic (CAT) (sub)domains [51].

DNA binding to Zn1, Zn3, and the WGR domain induces conformational changes in the helical domain (HD) and the ADP-ribosyl transferase (ART) subdomain of the CAT domain (Fig. 1, Video S1) by way of a network of inter-domain interactions [65,66,73]. Several key inter-domain interfaces mediate this allosteric signal [40,51,74]: (1) the

Zn1-Zn3 interface (Fig. 2A and B); (2) the Zn1-WGR-HD interface (Fig. 2A and C); (3) the HD-WGR-Zn3 interface (Fig. 2A and D); and (4) two HD-ART interfaces (Fig. 3A and B). See Videos S2 and S3 for an additional view of these critical interfaces.

4.2.1. Zn1-Zn3 interface

The Zn1-Zn3 interface forms when DNA binds Zn1, thereby exposing a Zn1 surface that subsequently binds Zn3 [49,75]. This interface, which primarily consists of the Zn1 C-terminal helix and the Zn3 N-terminal helix (Fig. 2A and B) [51], is close to but not in direct contact with the bound DNA (per the 4DQY crystal structure [51]). Mutagenesis studies suggest that the Zn1-Zn3 interface is critical for the allosteric communication that activates DNA-dependent catalytic activity, but that the interface does not influence DNA binding itself [51,66,72,75]. Mutations that disrupt major contacts between Zn1 and Zn3, such as R78 (Zn1) and W246 (Zn3), W79 (Zn1) and K238 (Zn3), etc. [72], decrease catalytic activity without disrupting DNA binding (Fig. 2B) [51,66,72,75]. For example, the conservative mutation K97R (Zn1) and the non-conservative mutations L77 P (Zn1) and K249E (Zn3) still bind DNA [76], but with PARP1 catalytic activity <0.5 % that of wild-type. Similarly, the R78A (Zn1) and W79A (Zn1) mutations result in markedly decreased catalytic activity, but bind DNA nearly as well as the wild-type [66]. And the W246A (Zn3) mutant has virtually no catalytic activity [51,75], likely because it cannot form the DNA-dependent Zn1-Zn3 interaction [75].

4.2.2. Zn1-WGR-HD interface

The Zn1-WGR-HD interface (Fig. 2A and C) also regulates DNA-dependent PARP1 catalytic activity [51,67] through contacts with DNA, Zn1, and the CAT-domain HD α E helix (Fig. 2C) [51]. The WGR domain interacts with DNA via the W589 (WGR) residue, which stacks

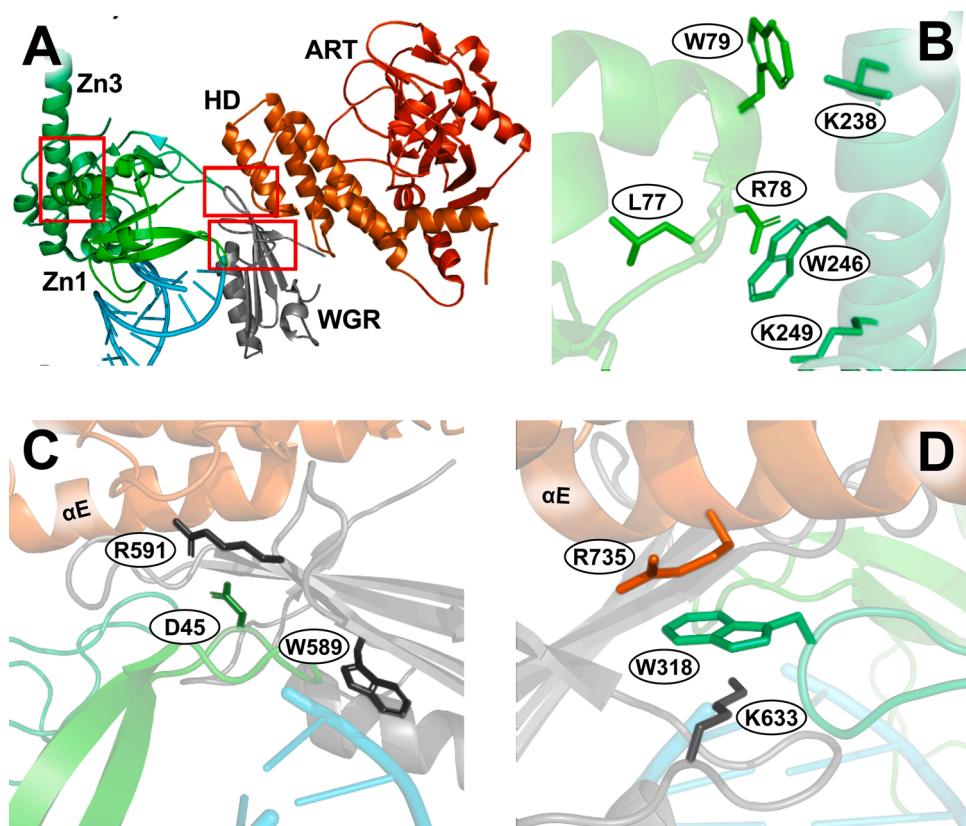


Fig. 2. Interdomain interfaces. Select residues are shown as sticks. (A) PARP1 (PDB: 4DQY) with red boxes encompassing three critical interdomain interfaces. (B) The Zn1-Zn3 interface. K97 (Zn1) is omitted, as it was not resolved. (C) The Zn1-WGR-HD interface. (D) The HD-WGR-Zn3 interface. See Ref. [51] for more details, and Video S2 for an additional review of these PARP1 interfaces.

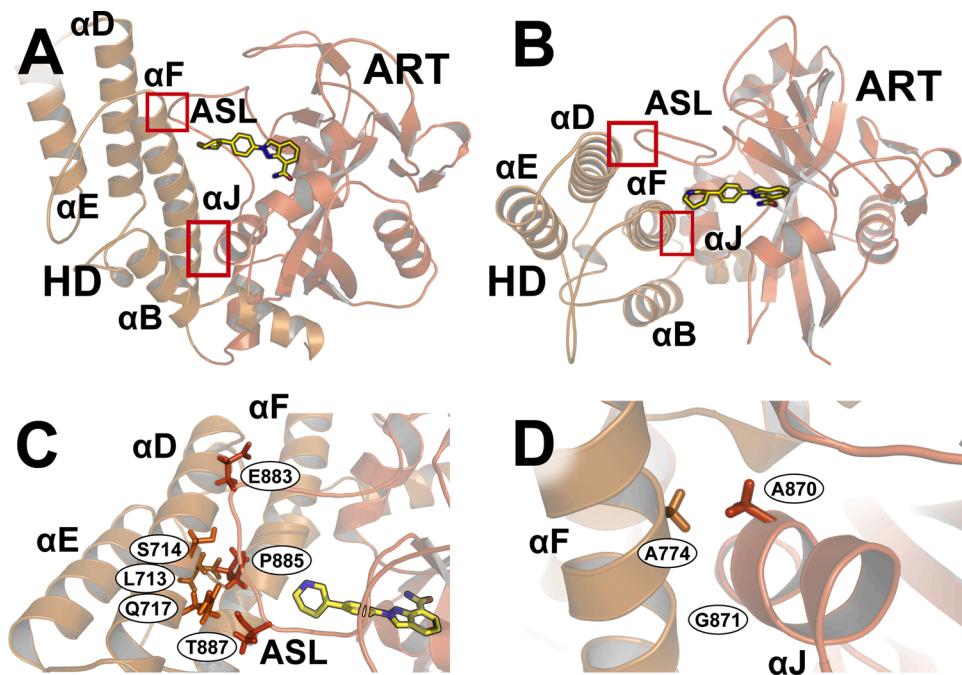


Fig. 3. Critical CAT interfaces (4R6E:A). Select residues are shown as sticks. (A) and (B) Two views of the interfaces between the HD (light orange) and ART subdomain (brownish orange), marked with red boxes. The PARPi niraparib (yellow sticks) binds the NI site. (C) The interface between the HD α D helix and the ART ASL. (D) The interface between the HD α F helix and the ART α J helix. Figure adapted with rights and permissions from Ref. [74]. See Video S3 for an additional review of these interfaces.

with the ribose sugars of the DNA 5' strand [51]. Visual inspection suggests that the Zn1 interacts with the WGR domain via an electrostatic interaction between D45 (Zn1) and R591 (WGR). The WGR in turn interacts with the HD α E helix in large part via non-specific hydrophobic interactions involving I562, V560, L641, and Y639 on the WGR, and L739 on the α E. As expected, mutations that disrupt these inter-domain interactions impact catalytic activity. The R591A (WGR) mutation disrupts PARP1 autoPARylation, as do the D45A (Zn1) and Q40A (Zn1) mutations [51,66,67].

4.2.3. HD-WGR-Zn3 interface

The HD-WGR-Zn3 interface also plays an important role in DNA-dependent catalytic activation and inter-domain communication. Aside from its interactions with the WGR domain (mentioned above), the HD α E helix also interacts with the extended loop of the Zn3 zinc-ribbon fold. The W318 (Zn3) residue, located on a loop that interacts with the R735 (HD) and K633 (WGR) residues, is at the center of this interface (Fig. 2A and D) [51]. W318R and W318A mutations reduce PARP1 PAR synthesis without impairing localization to DNA strand breaks [51,66,74], likely by disrupting inter-domain allosteric communication over the interface [51,74]. Similarly, the R735A (HD) and K633A (WGR) mutations also reduce PARylation activity [51,66].

4.2.4. HD-ART interfaces

Within the CAT domain, two critical interfaces between the HD and the ART subdomain profoundly impact PARP1 catalytic activity [74]: the α D-active site loop (ASL) interface and the α F- α J interface (Fig. 3). In the absence of DNA, the α D-ASL interface anchors the HD and the ART subdomain together [74,77], and the fully folded HD inhibits catalytic activity by blocking NAD⁺ access to the catalytic pocket—so much so that PARP1 constructs lacking the HD have DNA-independent activity equal to that of the DNA-bound wild-type protein [74]. When PARP1 binds to a DNA break, the specific assembly of regulatory domains (Zn1, Zn3 and WGR) creates a network of new interactions with the CAT domain that are mediated/propagated through the HD [74]. This allosteric signal causes select HD helical regions (α B, α D, and the C-terminal of α F) to unfold [74], disrupting the otherwise auto-inhibitory influence of the HD on the ART α J (Fig. 3A, B, and D; Video S3) [74]. The disruption opens the catalytic pocket and allows NAD⁺ to bind [74].

Mutations that disrupt the α F- α J interface (e.g., A774L and A774S on α F; A870 L, A870S, A871 L, and A871S on α J; etc.) partially decouple PARP1 catalytic activity from DNA binding. These mutations result in 3- to 10-fold increases in PARP1 DNA-independent activity (i.e., PARP1 catalytic activity when not bound to DNA), compared to the wild-type [74]. Some mutations that disrupt the α D-ASL interface (e.g., S714A and Q717A on the α D helix; P882 G, P885S, P885 G, and the quadrupole-substitution mutant E883A/A884S/P885S/V887A on the ASL) reduce DNA-dependent activity, with minimal impact on DNA binding and DNA-independent (basal) activity [74]. Mutations at L713 (L713 F/L713A; α D) are notable exceptions that increase DNA-independent activity [51,74,78].

4.3. PARP1 catabolizes NAD⁺ to perform ADP-ribosylation

Having discussed how PARP1 binds DNA and the allosteric communication that links DNA binding to catalysis, we now describe the catalytic mechanism itself. The highly conserved PARP1 CAT domain is located on the C-terminal region of the protein and includes the ART subdomain. This subdomain contains the enzymatic pocket, which binds NAD⁺ and catalyzes ADP-ribosylation (Fig. 1, Video S1) [65,66]. The catalytic pocket also binds all clinically approved PARP1 inhibitors (Fig. 1) [65], and “PARP1 inhibition” is most often measured in terms of PARP1 catalytic activity [65].

4.3.1. PARP1 catalytic mechanism

PARP1 ADP-ribosylation begins once NAD⁺ binds the catalytic pocket (Video S4) [40,49]. The pocket houses a highly conserved catalytic triad consisting of H862, Y896, and E988 (Fig. 4A), which are required for catalytic activity [49]. NAD⁺ interactions with these residues are well characterized because the CAT domain has been co-crystallized with benzamide adenine dinucleotide (BAD), a non-hydrolyzable NAD⁺ analogue (PDB: 6BHV; Fig. 4A [77]). H862 forms a hydrogen bond with the NAD⁺ 2' adenine-ribose hydroxyl group [49,79–81]; Y896 and Y907 interact with the nicotinamide moiety via π - π stacking and hydrophobic interactions, respectively (Fig. 4A) [79]; and E988 forms a hydrogen bond with the 2'-hydroxyl group of the nicotinamide ribose, which orients NAD⁺ as required for nucleophilic attack (Fig. 4A) [79]. This attack both catabolizes NAD⁺ and covalently

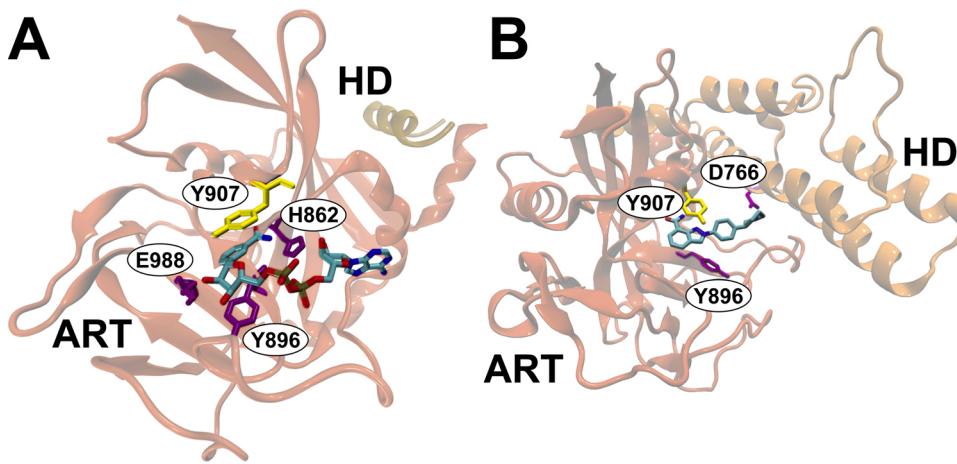


Fig. 4. Catalytic binding site. (A) BAD (sticks), an NAD⁺ analog, bound to the catalytic pocket (PDB: 6BHV:A). The catalytic triad (purple) binds to and catabolizes NAD⁺. Y907 and the catalytic-triad residue Y896 position the benzamide moiety via π - π stacking interactions. NAD⁺ likely binds the catalytic pocket in a similar orientation. (B) Niraparib (cyan sticks) bound to the catalytic pocket (PDB: 4R6E:A). Niraparib forms contacts with both the HD (light orange) and the ART (reddish orange). Select residues that interact with niraparib are shown as colored sticks.

attaches the resulting ADP-ribose to the acceptor protein or growing PAR chain [79].

The literature often divides the catalytic pocket into two subsites (Video S4). The first, called the donor site, includes three subregions that bind to and position the NAD⁺ “donor” molecule [79]: the nicotinamide-binding (NI), adenine-ribose-binding (ARB), and phosphate-binding (PH) sites. The NI site stabilizes the NAD⁺ nicotinamide moiety and is defined by the catalytic triad residues Y896 and E988, as well as Y907 [79]. The ARB site stabilizes the NAD⁺ adenine-ribose moiety and is defined by the catalytic triad residue H862, as well as D770, S864, G876, and R878 [79]. Finally, the PH site, which consists of E763 and D766, stabilizes the NAD⁺ pyrophosphate group [79]. The majority of all PARP1 inhibitors bind to PARP1’s donor site (e.g., as in Fig. 4B) [49,82].

The second catalytic-pocket subsite, called the acceptor site, binds and positions the pyrophosphate group of the “acceptor” amino acid or PAR chain to be ADP-ribosylated (Video S4) [79]. The acceptor site consists of H826, M890, K903, L985, and Y986 [49,79] and is thought to influence the branching properties of the PAR chains [49,82].

4.3.2. Influence of HPF1 on PARP1 catalysis

Recent work has identified histone PARylation factor 1 (HPF1) as a critical PARP1 binding partner that impacts the PARP1 catalytic mechanism [83]. Histones are among the primary targets for PARylation by PARP1 [83,84]. The PARP1 C-terminal domains (WGR/CAT) bind to histone H4 in the nucleosome, leading to long-term ADP-ribosylation that “loosens” the chromatin [64]. This loosening improves transcription-machinery access to DNA and enables DNA-damage repair and DNA replication [64].

HPF1 plays an essential role in facilitating PARP1 ADP-ribosylation of histones [83,84]. Like PARP1, HPF1 is recruited to sites of DNA damage. This recruitment requires the PARP1 protein itself but not PARP1 enzymatic activation, suggesting that HPF1 recruitment is independent of PARylation [85,86]. When the PARP1 ART subdomain is in an activated state (i.e., the otherwise auto-inhibitory HD subdomain has locally unfolded and NAD⁺ is bound; see Section 4.2.4), the HPF1 C-terminal region binds to the PARP1 CAT/ART subdomain [84–86]. In this bound state, a portion of HPF1 occupies part of the PARP1 active site, contributing key residues to a new composite binding pocket [84].

The newly formed composite pocket differs from the original PARP1-only site in three fundamental ways. First, though PARP1 normally ADP-ribosylates many amino acids (e.g., aspartate, glutamate), the composite site acts predominantly on serine [83,85,87,88]. This switch in specificity is important because recent evidence suggests PARylation in the context of DNA damage occurs primarily on serine residues [84,89]. Crystal structures show that HPF1—not PARP1—contributes the active-site catalytic base (HPF1 E284) that deprotonates serine hydroxyl

groups [83,85], thus enabling the subsequent nucleophilic attack required for ADP-ribosylation [84].

Second, the electrostatics of the composite active site may promote histone association [84]. Like histones, the PARP1-only site and surrounding surfaces are positively charged. But the PARP1/HPF1 composite site has an overall negative charge that is arguably more compatible with histone binding [84].

Third, HPF1 binding to PARP1 limits the autoPARylation typical of PARP1 activity [84,85]. Bound HPF1 occupies roughly the same position relative to PARP1 that would otherwise be occupied by the PARP1 AD domain. The AD domain includes many PARP1 automodification sites, so HPF1 likely limits hyper-automodification by displacing the AD domain from its location near the catalytic site [84,86]. Reduced autoPARylation may further encourage PARP1 association with DNA and, indirectly, DNA-associated histones, because PAR chains are negatively charged and thus promote PARP1 dissociation from negatively charged DNA [34,64]. The pharmacological impacts of the HPF1/PARP1 interaction are discussed further in Section 6.2.

4.4. Release from DNA is regulated by interdomain interactions

PARP1 allostery has historically been discussed in terms of the influence that DNA binding has on catalytic activity [29,51,65], but recent research has also examined the influence of the PARP1 CAT domain on DNA unbinding [77,90]. In this section, we discuss PARP1/DNA dissociation and how PARP1 inhibitors might act by interfering with the dissociation mechanism.

Some catalytic-pocket ligands trigger an allosteric mechanism that may immobilize PARP1 on DNA [77,90], a phenomenon called “trapping” [77,91–96]. It has been proposed that trapping PARP1 on DNA prevents DNA repair proteins from accessing strand breaks, stalls and/or collapses replication forks, and results in cell-cycle arrest or apoptosis [91]. However, more recent experiments using quantitative live-cell imaging and fluorescence recovery after photo-bleaching suggest that PARP1 molecules in fact undergo rapid exchange at the locations of DNA strand breaks [97], consistent with observations that single PARP1 molecules have increased constrained motion (i.e., DNA sliding) when bound to the inhibitor olaparib [92]. The role that trapping plays in the mechanism of current PARP1 inhibitors thus remains controversial; indeed, among PARP1 inhibitors used clinically, overall toxicity does not correlate clearly with the degree of presumed trapping [98].

4.4.1. Catalytic-pocket ligands may trigger trapping via allostery

Some have claimed that different catalytic-pocket ligands impact “trapping” to varying degrees [77,94,95]. The degree of chromatin retention appears to be related to each ligand’s ability to (de)stabilize the HD [90]. Those that destabilize the HD (e.g., the NAD⁺ analog BAD)

allosterically strengthen PARP1's affinity for DNA and consequently have strong trapping capacity [77,90]. In contrast, those that stabilize the α B and α F helices of the HD weaken DNA binding affinity and release PARP1 from DNA [90].

For example, the catalytic-pocket ligand EB-47 forms contacts with D766 and D770, two residues located on the HD α F helix [90]. These contacts destabilize the helix, possibly leading to PARP1 "trapping" on DNA [90]. But the D766A/D770A double mutant disrupts the contacts, thereby preventing trapping [90]. In contrast, the PARP1 inhibitor veliparib does not form contacts with the HD when bound to the catalytic pocket (Fig. 5A) and so is apparently less effective at trapping wild-type PARP1. But UKTT-15, a veliparib-derived inhibitor that does form contacts with the N-terminal portion of the HD α F helix (Fig. 5B; Video S3), is more effective at DNA trapping and more toxic to BRCA-deficient cells, even though veliparib and UKTT-15 inhibit PARylation to similar degrees [90].

Small-molecule (de)stabilization of the HD triggers allosteric inter-domain communication between the CAT domain and the DBD [90], through the HD-WGR-Zn3 and Zn1-WGR-HD interfaces. The W318R (Zn3) and R591C (WGR) mutations, which disrupt the HD-WGR-Zn3 and Zn1-WGR-HD interfaces, respectively, appear to prevent EB-47 trapping activity. Mutations such as these likely have clinical significance. The R591C mutation was recently identified in a patient with ovarian cancer who had developed resistance to the PARP1 inhibitor talazoparib [90,99].

4.4.2. PARPi classification system

A recently developed PARPi classification system groups PARP1 inhibitors into three types based on their allosteric effects [90]. This system is based on the hypothesis that some inhibitors can promote PARP1 retention on DNA. Type I inhibitors are those that allegedly promote PARP1 trapping through allosteric regulation, resulting in long DNA-retention times and high toxicity [90]. BAD, UKTT-15, and EB-47 are good examples of this class because they apparently promote PARP1 "trapping" via contacts that disrupt the HD (Fig. 5B; Video S3) [90]. Type II inhibitors are neither pro-retention nor pro-release (i.e., relatively allosterically neutral), resulting in moderate DNA-retention times and toxicity [90]. Examples include talazoparib and olaparib, which successfully inhibit PARylation without much impact on DNA trapping [90]. Finally, type III inhibitors promote PARP1 release through allosteric mechanisms, resulting in short DNA-retention times and lower toxicity [90]. Rucaparib, niraparib, and veliparib are good example of type III inhibitors. They stabilize the α B and α F helices of the HD while simultaneously promoting increased α C/ α D-helix dynamics [90]. This influence ultimately increases PARP1 dissociation from DNA [90]. Aside from these allosteric considerations, other factors may also contribute to

PARP1 trapping in cells [90,100,101], including potency of inhibition, PARP-1 affinity, and compound half-life, though there is controversy [96].

5. PARP1 in cancer and cancer treatment

In this section, we will (1) discuss the prevalence and available treatments for patients with HR-deficient cancers; (2) describe the mechanisms that make PARP1 drug inhibition lethal to HR-deficient cancers; (3) overview current clinically approved PARP1 inhibitors; (4) document common PARPi-resistance mechanisms; and (5) describe the shortcomings of current pharmaceutical options as well as strategies for improvement.

5.1. HR-deficient cancers: prevalence and standard-of-care management

Breast, ovarian, prostate, and pancreatic cancers are frequently HR deficient [102–105], often due to mutations in the BRCA1/2 genes [102–104,106], which encode breast-cancer tumor-suppressor proteins that play critical roles in HR DNA repair. Since the 1970s, surgical cytoreduction together with chemotherapy have been the primary treatments for HR-deficient cancers. But traditional chemotherapies do not leverage the specific biology of HR deficiency to achieve a more targeted approach.

More recent drugs that inhibit PARP1 are effective against many types of cancer [21,28–31,42,93,107]. These inhibitors tend to be more toxic to HR-deficient cancer cells than to non-cancerous cells and so have the potential for fewer side effects. Several studies have shown that PARP1 inhibitors are effective as monotherapies (i.e., standalone treatments) and co-treatments alongside other chemotherapeutics (i.e., combination therapies) [21,28–31,42,93,107].

5.2. HR-deficient cells and PARPi sensitivity: synthetic lethality

PARP1 inhibition is an excellent strategy for treating multiple cancers, in part because PARP1 plays well-documented roles in multiple DNA repair pathways [28–31]. PARP1 inhibitors are particularly effective against HR-deficient cancer cells because the HR pathway has a synthetically lethal relationship with PARP1 (i.e., a cell can survive a deficiency in HR repair or PARP1 function, but not in both, due to some functional redundancy) [21,35,61,107,108]. PARP1 inhibition is in fact the first successful example of a synthetic-lethal approach in pharmaceutical treatment [61].

HR-deficient cancer cells lack an important DSB repair pathway, but they remain viable by exploiting the complementary functions of non-HR repair pathways (e.g., the BER and NHEJ pathways) [35].

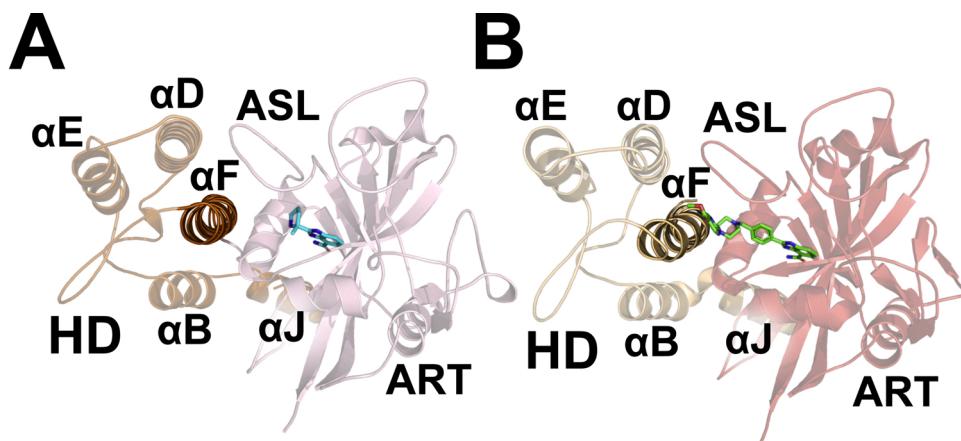


Fig. 5. Differences in veliparib and UKTT-15 binding to the CAT domain. (A) Veliparib (cyan, 2RD6:A) does not interact with the HD. (B) UKTT-15, a veliparib analog (green, 6VK0:A), displaces the HD α F helix. See Video S3 for further discussion.

Consequently, PARP1, which is responsible for recruiting the BER [91] and alt-NHEJ [23] pathways, is often upregulated in HR-deficient cancer cells [34,109], and HR-deficient cells tend to be hypersensitive to PARP1 inhibition [34,109]. Impaired HR combined with PARP1-inhibited BER/alt-NHEJ leads to an accumulation of DSB lesions. These lesions are particularly detrimental to actively replicating cells (e.g., tumor cells) because unrepaired breaks often result in daughter cells that lack essential genes. In contrast, wild-type (HR-proficient) cells are insensitive to PARP1 inhibitors because they can still repair DNA damage via high-fidelity HR [21,35,61,107,110].

5.3. Clinically approved PARP1 inhibitors (PARPi)

Currently, there are four FDA-approved PARP1 inhibitors (olaparib, rucaparib, niraparib, and talazoparib) for treating HR-deficient cancers (Fig. 6) [21,28–31,40,42,93,107], and several more are in varying stages of clinical trials [28–31]. Although some PARP1 inhibitors are used as standalone cancer treatments, they are commonly paired with other treatments such as DNA-damaging chemotherapies or radiation [42,93,107]. For example, olaparib, the first FDA-approved PARP1 inhibitor [30], has been used as both a monotherapy and in combination with other therapies [30,111,112]. It was first approved in 2014 for treating ovarian cancers with BRCA1/2 mutations [30] but is now also used to treat some HR-defective breast [113], prostate [114], and pancreatic [115] cancers.

All current PARP1 inhibitors used in the clinic bind to the PARP1 catalytic pocket and act as competitive inhibitors of NAD⁺ [90]. They share structural similarities with nicotinamide (Fig. 6) [107] and so form similar protein-ligand interactions [65,74,116–123]. For instance, many PARP1 inhibitors, including the NAD⁺ analog BAD and the drug niraparib (Fig. 4), form π-π stacking interactions with the PARP1 Y907 residue [65,74,117–124].

Aside from impairing PARylation, PARP1 inhibitors may also be lethal to HR-deficient cells because of their varying abilities to “trap”

PARP1 on DNA [90,125,126], which could potentially cause stalling and/or replication-fork collapse, resulting in cell-cycle arrest or apoptosis [91]. As evidence that competitive inhibition alone cannot explain the toxicity of PARP1 inhibitors, we note that cellular sensitivity to PARPi decreases when PARP1 expression is reduced via siRNA knockdown [96]. If competitive catalytic inhibition were entirely responsible for PARPi toxicity, one would expect that siRNA knockdown/knockout—which similarly reduces or even abolishes that activity—would be similarly toxic. That this is not the case suggests that PARP1 inhibitors confer additional toxicity through secondary mechanisms. DNA trapping is one possible, albeit controversial, explanation.

5.4. PARPi resistance mechanisms

Because PARP1 is involved in multiple complex biological pathways, many possible resistance mechanisms enable cancer cells to compensate for PARP1 inhibition. In this discussion we will focus on resistance mechanisms known to affect HR-defective cells: (1) HR reversion/increased HR capacity; (2) altered NHEJ capacity; (3) corrected replication forks; (4) decreased intracellular PARPi concentrations; and (5) modified PARP1 expression, activity, allosteric regulation, PARylation, and/or PARPi binding.

5.4.1. HR reversion and increased HR capacity

Many proposed and clinically observed PARPi resistance mechanisms involve increased HR capacity [35,127]. HR-deficient cells are sensitive to PARP1 inhibitors precisely because they have an impaired HR DNA repair pathway and so cannot survive the additional inhibition of PARP1-implicated repair pathways [35,127]. Consequently, partial or complete restoration of HR capacity results in PARPi resistance [35,127]. This section will discuss three mechanisms that increase HR capacity: (1) restoring HR-proficiency through secondary reversion mutations, (2) increasing HR protein availability, and (3) altering regulators of HR repair.

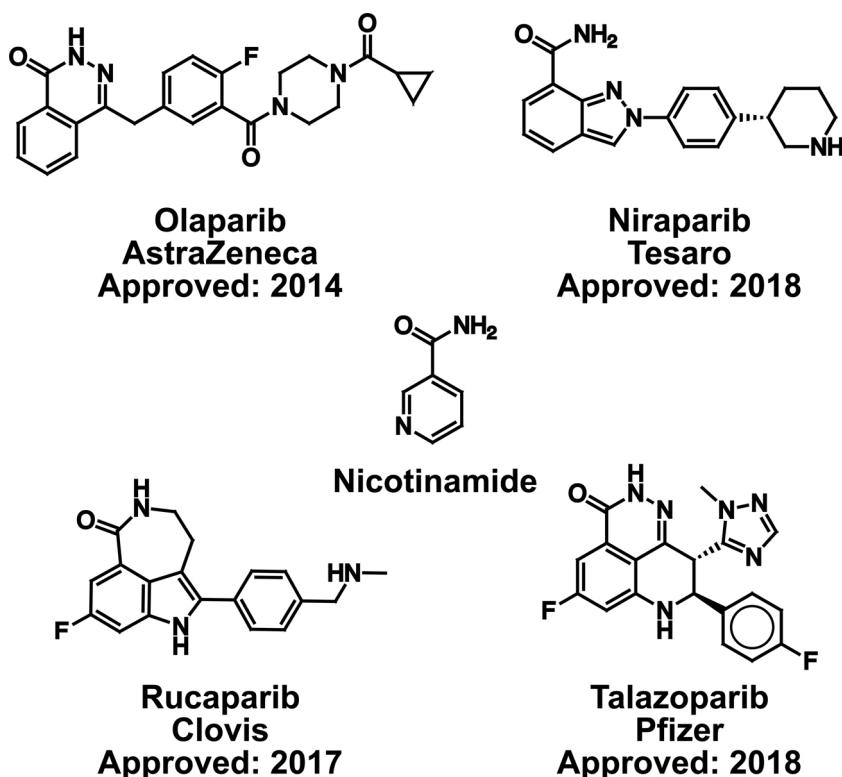


Fig. 6. The four FDA-approved PARPi. Chemical structures are shown together with the generic names, companies, and years of FDA approval. Nicotinamide, a NAD⁺ moiety, is shown to illustrate the chemical similarities between the PARPi and the endogenous ligand.

5.4.1.1. Secondary reversion mutations that restore HR proficiency can cause PARPi resistance [127]. HR-deficiencies are frequently caused by single-nucleotide mutations, short insertions, and frameshift deletions that disrupt the BRCA1/2 genes [102,103,127]. Secondary mutations that restore BRCA function can desensitize previously BRCA-deficient cells to PARP1 inhibitors, per multiple clinical observations [35,127, 128]. The restoration of these genes speaks to the selective pressure that PARP1 inhibitors exert on cancer cells [127]. Unfortunately, reversion mutations in BRCA1/2 are often associated with resistance to platinum-based chemotherapies [129] as well, though treatment with taxanes may still be effective in such circumstances [130].

5.4.1.2. Increased availability of HR proteins can also cause PARPi resistance [35,131–133]. Several factors influence the expression of these proteins. For instance, PARP1 itself negatively regulates BRCA2 expression by binding to the silencer-binding region of the brca2-promoter [35,131]. By inactivating and possibly “trapping” PARP1 on DNA, PARP1 inhibitors could thus indirectly increase BRCA2 expression, contributing to PARPi resistance [35,131]. Other factors also impact BRCA1 expression. For example, reduced levels of the microRNA miR182, which negatively regulates BRCA1 expression, can also increase BRCA1 protein production and so can lead to PARPi resistance [35,132,133].

Altered expression of other HR-pathway proteins also contributes to resistance. Overexpression of RAD51, a recombinase and important HR protein, is one example [35,134,135]. RAD51 is frequently overexpressed in BRCA1-deficient tumors and is associated with partially restored HR repair [35,134–137]. RAD51 overexpression has been observed in a rucaparib-resistant high-grade ovarian carcinoma, as well as colon carcinoma cells that were resistant to a PARPi administered with the alkylating chemotherapeutic temozolomide [35,134,135].

5.4.1.3. Proteins that regulate HR repair also influence HR capacity. These proteins include 53BP1 and ataxia telangiectasia mutated (ATM) [35,127,136,138,139]. 53BP1 is similar to BRCA1 in that it modulates HR and NHEJ repair, but it has the opposite effect on these pathways. Whereas loss of BRCA1 results in decreased HR repair and increased NHEJ repair, loss of 53BP1 results in increased HR repair and decreased NHEJ repair [35,127,136,138]. This increased HR repair can contribute to PARPi resistance in previously HR-deficient cells [35,136,138]. 53BP1 acts on the HR-repair pathway via the ATM kinase, which triggers a kinase cascade in response to DNA damage that ultimately promotes HR repair [136,138]. Increased ATM activity can also confer PARPi resistance [35,136,138]. Some have proposed combination therapies that include PARP1 and ATM inhibitors to circumvent these resistance mechanisms [139].

5.4.2. Altered NHEJ capacity

Changes to an HR-defective cell’s NHEJ capacity can also cause resistance to PARP1 inhibitors [21,26,35,127]. Because NHEJ repair is regulated by 53BP1 and BRCA1, alterations to these proteins can impact NHEJ capacity [35,127,136,138]. Factors that alter the expression or function of NHEJ proteins such as DNA-PK and Ku proteins can also impact NHEJ capacity and consequently cellular sensitivity to PARPi [21,35,127]. Both the downregulation and upregulation of NHEJ proteins can drive chromosomal instability, resulting in PARPi resistance [26,35]. The consequences of up/downregulated NHEJ capacity are reviewed in more depth in refs. [26,35].

5.4.3. Corrected replication forks

Some PARP1 inhibitors cause PARP1 to stall replication forks when bound to DNA [91]. BRCA1/2 proteins are involved in DNA-strand protection at stalled forks [32], so BRCA-deficient cells are sensitive to fork collapse and nascent-strand shortening [127,140,141]. Consequently, proteins that stabilize the replication fork can contribute to

PARPi resistance [141]. For example, the nucleosome remodeling factor chromodomain-helicase-DNA binding protein 4 (CHD4) is expressed at lower levels in some chemotherapy-resistant BRCA2-mutant cancer cells, even though those cells remain deficient in RAD51-dependent HR repair [141]. BRCA2-deficient cells with reduced CHD4 can better protect nascent replication tracts and are therefore resistant to PARP1 inhibitors [141,142].

5.4.4. Decreased intracellular PARPi

Factors that reduce intracellular PARPi concentration can also impact PARPi efficacy. P-glycoprotein, an ATP-binding cassette (ABC) drug efflux transporter, is largely responsible for regulating these concentrations [35,127,143–146]. Reduced P-glycoprotein levels tend to increase sensitivity to PARP1 inhibitors in BRCA1-deficient mouse models [143,144]. Additionally, P-glycoprotein inhibition re-sensitizes some BRCA-deficient, PARPi-resistant cells to PARPi [143,145]. This resistance mechanism has been studied mainly in animal and cell models, so further research is needed [35,127,143–146].

5.4.5. Altered PARP1 capacity

Factors that impact PARylation and potentially PARP1 “trapping” on DNA can contribute to PARPi cytotoxicity in HR-deficient cells [90,125, 126]. Alterations to either of these processes, as well as to PARP1 expression levels, can lead to PARPi resistance.

5.4.5.1. Changes to the PARP1 catalytic site (where PARylation takes place) are particularly impactful. For example, the proto-oncogene receptor tyrosine kinase c-Met, which is commonly overexpressed in many forms of cancer, alters the PARP1 catalytic site by phosphorylating Y907 (Video S4) [147,148]. Y907 forms π-π stacking interactions with both NAD⁺, the endogenous substrate, and all clinically approved PARP1 inhibitors [148]. Phosphorylated Y907 (pY907) has increased catalytic activity, but weakened binding affinity for multiple PARP1 inhibitors [147,148]. c-Met upregulation can therefore confer broad resistance [148]. Indeed, pY907 is known to confer resistance to veliparib, olaparib, and rucaparib [148].

5.4.5.2. Changes to other proteins responsible for regulating PARylation also contribute to PARPi resistance. PAR glycohydrolase (PARG), a protein that degrades PAR chains, is one such protein [127,149]. Factors that reduce PARG activity prevent PAR-chain degradation, potentially compensating for the reduced PARylation caused by PARP1 inhibition. This resistance mechanism was first discovered in a screen of BRCA2-deficient mouse cells with partially rescued PARP1-dependent DNA damage signaling [127,149]. Knockdown of PARG by shRNAs in human cancer cells with BRCA1 and BRCA2 deficiencies (SUM149PT and DLD-1, respectively) caused olaparib resistance [149]. Low PARG expression levels have also been measured in a subset of human triple-negative breast tumors and serous ovarian carcinomas [149].

5.4.5.3. Mutations that disrupt the allosteric communication allegedly required for PARP1 “trapping” on DNA may also contribute to PARPi resistance. At least one mutation—R591C (WGR) at the Zn1-WGR-HD interface—has been identified clinically in a talazoparib-resistant ovarian tumor. This same mutation appears to reduce PARP1 trapping [90,99]. Many additional lab-generated, interfacial mutations are known to alter PARP1 activity, PARPi inhibition, and/or PARPi trapping (see above), suggesting additional routes to PARPi resistance.

5.4.5.4. PARP1 expression levels and catalytic activity correlate with PARPi sensitivity. For example, PARPi-resistant colorectal-carcinoma HCT116 cells have markedly low levels of PARP1 expression [35,134]. Cancer cells with decreased PARylation activity but normal PARP1 expression levels also tend to be more resistant to PARP1 inhibitors [35, 150]. On the other hand, HR-deficient cells with higher levels of

PARylation tend to be more sensitive to PARP1 inhibitors [35,151].

6. Outlook

Because all clinically approved PARP1 inhibitors bind the same catalytic pocket with similar binding modes, they are likely subject to similar resistance mechanisms. Given this general vulnerability, there is a need for next-generation PARP1 inhibitors that can evade common resistance mechanisms. In this section, we will suggest several future pharmaceutical approaches to PARP1 inhibition.

6.1. Targeting alternative binding pockets

Given that many PARPi resistance mechanisms involve changes to the catalytic pocket where PARP1 inhibitors bind, identifying next-generation inhibitors that target other pockets is a promising pharmacological approach. New PARP1 inhibitors that do not rely on the same binding interactions as current inhibitors may overcome resistance mechanisms caused by PARP1 post-translational modifications or mutations.

There is good reason to suppose that allosteric ligands that bind outside the catalytic pocket could be successfully exploited in future drug-discovery campaigns. It is true that all known PARP1 inhibitors act as orthosteric inhibitors of PARylation, but some may also act as allosteric inhibitors that “trap” PARP1 on DNA [94,96]. The allosteric signal that links the CAT domain and the DBD must traverse several important inter-domain interfaces. Mutagenesis studies show that disrupting these interfaces can decouple catalytic activity from DNA binding (see above) [51,66,75]. Small-molecule drugs that similarly disrupt these interfaces by binding to adjacent pockets might also limit catalytic activity while preserving PARP1 trapping on DNA.

To provide further support for this pharmaceutical approach, we applied FTMap [152] to the 4DQY [51] PARP1 structure. FTMap is an algorithm that searches for druggable “hotspots” with favorable ligand-binding properties [152]. It identified five PARP1 hotspots (excluding those positioned on the co-crystallized DNA molecule, Fig. 7) [40].

6.1.1. Catalytic pocket

Two hotspots located in the CAT domain serve as positive controls because both occupy the catalytic binding site, where existing inhibitors are known to bind.

6.1.2. Zn1-WGR-HD interface

One hotspot is located near the Zn1-WGR-HD interface (Fig. 7A, bottom center; Video S4). PARP1 with mutations at this interface, such as D45A (Zn1) and R591A (WGR), has near-wild-type levels of DNA binding activity despite dramatically reduced catalytic activity [51,66,67]. Small molecules that similarly disrupt the interface could in theory have a similar impact.

The apparent volume of the hotspot-associated pocket (Fig. 7B, bottom center), as measured by the DoGSiteScorer algorithm [153], is also encouraging. The Zn1-WGR-HD pocket has roughly half the volume of the ligand-bound, 4R6E:A catalytic pocket [65] (595 \AA^3 vs. 1262 \AA^3), but its size is still well within the range of typical ligand-binding pockets ($100\text{--}1,000 \text{ \AA}^3$ [154]). This finding is important because larger and deeper pockets are typically considered more druggable [155].

6.1.3. Zn1-Zn3 interface

Two hotspots located at the Zn1-Zn3 interface are close to each other and so can be considered a single site (Fig. 7A, on the left; Video S4). Disrupting the Zn1-Zn3 interface is also a promising pharmacological strategy, given that PARP1 with the W246A (Zn3) mutation has near-wild-type levels of DNA binding activity but much reduced catalytic activity [51,66,75]. Notably, the Zn1-Zn3 pocket (Fig. 7B, on the left) is slightly larger than even the Zn1-WGR-HD pocket (634 \AA^3 vs. 595 \AA^3), per DoGSiteScorer [153].

6.2. Targeting the PARP1/HPF1 composite catalytic site

The recently identified HPF1 protein (see Section 4.3.2) profoundly impacts the PARP1 catalytic site by contributing key residues that enhance serine ADP-ribosylation activity [83–85,87,88]. This new PARP1/HPF1 composite pocket is so radically altered that it is effectively a new pocket. The associated active-site changes present many new opportunities for drug discovery; indeed, recent work has shown that HPF1 has a substantial impact on the binding affinity of some PARP1 inhibitors (e.g., it increases the affinity of olaparib) [83]. On the other hand, it does not appear to affect the affinity of other inhibitors such as veliparib [83]. The role of HPF1 in regulating PARP1 activity has only recently been characterized [86], and the first structures of the PARP1/HPF1 complex were published in 2020 [87,88]. Consequently, the PARP1/HPF1 interaction has yet to inform the design of any clinically approved PARP1 inhibitor. Designing inhibitors that bind the composite PARP1/HPF1 catalytic site is a promising future direction.

6.3. Combining treatments to circumvent PARPi resistance

Aside from identifying novel PARP1 inhibitors, combining such inhibitors with other treatments will continue to be critical. PARP1 inhibitors are unlikely to overcome some resistance mechanisms, regardless of their specific mode of action (whether orthosteric/competitive or allosteric). As described above, examples of such resistance mechanisms include secondary BRCA1/2 mutations that restore HR function and factors that limit PARP1 expression.

Existing, clinically approved PARP1 inhibitors are often paired with other chemotherapeutics and/or surgical cytoreduction [21,28–31,42,93,107]. Combination therapies that target proteins/pathways involved in PARPi resistance can in many cases (re)sensitize cells to PARPi. Several studies have examined the synergistic effects of combining

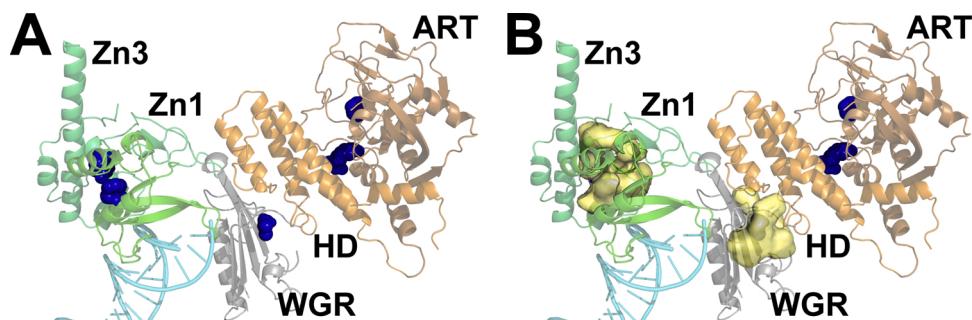


Fig. 7. FTMap-detected hot spots (PDB: 4DQY). (A) Molecular fragments used to identify hot spots are shown as blue spheres. There are two hot spots at the Zn1-Zn3 interface and two in the catalytic pocket. (B) The pocket volumes of the Zn1-Zn3 interface hot spot (top left) and the Zn1-WGR-HD interface hot spot (bottom center) are shown as transparent yellow surfaces, calculated using POVMEM2 [160]. See Video S4 for further discussion.

PARP1 inhibitors with inhibitors of ataxia-telangiectasia mutated and Rad3-related (ATR) kinase [156], c-Met [148], and histone deacetylase [157]. Interested readers may wish to consult Yi et al., who recently reviewed several additional PARPi combination therapies [158]. Further research is needed to identify which co-treatment strategies can optimally circumvent the many documented PARPi resistance mechanisms.

7. Conclusions

Though PARP1 has been the subject of intense study, much remains unknown about this complex, multi-domain protein with both orthosteric and allosteric mechanisms. Several clinically approved PARP1 inhibitors target the catalytic pocket, but future small-molecule ligands that act at other sites have the potential to evade known resistance mechanisms. Ligands designed to disrupt the various PARP1 allosteric mechanisms could serve as selective therapeutics for the treatment of PARPi-sensitive cancers.

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Transparency document

The Transparency document associated with this article can be found in the online version.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.dnarep.2021.103125>.

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